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POSSIBLE METHODS OF CLASSIFYING WHITE, YELLOW AND ORANGE STAPHYLOCOCCI,
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Possible Methods of Classifying White, Yellow and Orange Staphylococci.*

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INTRODUCTION.

STAPHYLOCOCCI were first found in pus by Pasteur [1] (1880). Ogston [2] confirmed Pasteur's work a year later (1881), and in 1883 Becker [3] was able to isolate staphylococci in pure culture. Rosenback [4] (1884) described *staphylococcus pyogenes*, dividing it into two varieties corresponding to the orange and white pigmentation, calling them var. *aureus* and var. *albus*. In 1908 the Winslows [5] based their classification upon growth, pigment production and liquefaction of gelatin.

Dudgeon [6] (1908) found *staphylococcus albus* commonly in normal tissue while *staphylococcus aureus* was usually obtained from pathogenic sources. He was interested in the interchangeability of these two varieties and worked upon a classification of these organisms, using glucose, lactose, maltose, glycerin, cane sugar, raffinose, erythrite, salacin, litmus milk and neutral red. He finally concluded that they all belonged to the same species.

Winslow, Rothberg and Parsons [7] (1920) studied 180 cultures of white and orange staphylococci to determine their action upon the sugars, glucose, lactose, sucrose, maltose, raffinose, mannitol, dulcitol, salacin and inulin. They used two different media, the dehydrated bacto nutrient broth prepared by

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the Digestive Ferments Company, and the peptone media of Clark and Lubs. They found that: "The action of the staphylococci upon glucose, maltose, sucrose and lactose would seem to offer a possible basis of classification, although the marked differences due to the effect of the medium would suggest the use of this property as a differential test might prove of doubtful value."

They were able to divide the organisms into three main groups. Group I, organisms fermenting all four sugars; group II, organisms fermenting glucose, maltose and sucrose, but not lactose. In group III they classified all the rest of the strains and stated that it was a "highly heterogeneous agglomeration."

They found that "gelatin liquefaction was slightly but distinctly more common among the active fermenters," and that "white and orange pigments were fairly evenly divided among the various fermentative groups with a slightly greater preponderance of vigorous fermenters in the orange than in the white group." Their tests for indol were all negative and nitrate broth gave almost uniformly positive results showing reduction.

Winslow, Rothberg and Parsons, after this extensive work upon various sugars, nitrates, indol chromogenesis and gelatin liquefaction, state that: "Fundamentally we are inclined to agree with Dudgeon in considering the whole group a reasonably homogeneous one, and it seems clear the central type of the whole genus is the orange-pigment forming, vigorously fermenting, gelatin liquefying, somewhat actively pathogenic *St. aureus*. As we depart from this type there is a progressive weakening of the various biochemical activities of this more vigorous form. The loss of one characteristic of the *St. aureus* type tends in some degree to be associated with the loss of others. Thus the white chromogens are less actively pathogenic than the orange forms, less actively gelatinolytic and slightly less vigorous in fermentation action. The forms which fail to liquefy gelatin also tend to be less active fermenters than the liquefiers."

The object of the present paper was to obtain white, yellow and orange staphylococci from as many different sources as possible and to see whether the group would lend itself to rational or satisfactory subdivision making use of fermenta-

tion, pigmentation, hemolysis, proteolysis on milk agar plates, liquefaction of gelatin, blackening of lead acetate agar, and the determining of limiting hydrogen ion concentrations of each strain in dextrose broth. I hoped to see if there was a correlation of any of these with source and pathogenicity.

In order to do this, I have subdivided this work under six headings, as follows:

1. Assuming as Dudgeon that staphylococci seemed to be one species and disregarding the characteristic of pigment production and liquefaction of gelatin, is it possible to subdivide staphylococci in general upon a basis of fermentation of carbohydrates. In determining data for this question, I have asked myself to note the following questions: Does the classification by fermentation reaction offer any correlation with pigment production, liquefaction of gelatin, with pathogenicity, with source? and, Is there a correlation between rapidity of fermentation and of pigment production and pathogenicity as suggested by Winslow?

2. After studying staphylococci as a whole from the standpoint of fermentation reactions, it was next decided to assume pigmentation as the primary differentiation into subgroups of white, yellow and orange staphylococci and attempt the subdivision of each of these by means of fermentation reaction. The borderline yellows and orange pigment producers were placed in their respective groups of yellow or orange.

3. The next step was to assume, as before, pigmentation as a primary differentiation into white, yellow and orange staphylococci then to attempt a subdivision of each of these by means of blood agar plates, placing the hemolizers and nonhemolizers in separate groups as has been done for streptococci, these were again subdivided upon the basis of fermentation reactions. In the work on hemolysis, a comparative study was made using different kinds of blood, such as rabbit, sheep and human.

4. A similar study of staphylococci in which pigmentation was made use of for primary subdivision of each group, subdivided again in accordance with the ability of various strains in that group to produce proteolysis upon milk agar plates. This gave proteolytic and nonproteolytic subdivision. These were further divided upon the basis of fermentation. It was necessary to study the reationship between reaction of media and degree of proteolysis in obtaining data for this work.

5. To study the ability of the various staphylococci to produce hydrogen sulphide, all staphylococci were first inoculated into both one per cent peptone broth agar containing lead acetate, and three per cent peptone broth agar containing lead acetate to see whether there was any correlation between the blackening of lead acetate and any other characteristics. I might say there was noted apparently a correlation between pathogenicity and blackening of three per cent peptone lead acetate agar.

6. Lastly, it was thought worth while to determine the limiting hydrogen ion concentrations of all these various staphylococci in dextrose dipotassium phosphate broth to see whether there exist high and low

ratio groups and whether these correlate with any other characteristics and data.

In all, 75 strains of staphylococci were studied. These were obtained from pathological conditions, in various foods and three strains from the American Museum of Natural History. My tentative definition for staphylococci was cocci in which the division was in two planes giving rise to flat sheets of cells and irregular masses.

TECHNIQUE.

All organisms used in this work were freshly isolated and were first grown upon agar, + 1 to phenolphthalein, then inoculated into plain broth to determine morphology.

In studying fermentation, the organisms were inoculated into one per cent sugar broth solutions of dextrose, lactose, saccharose, mannite, maltose, salacin, dulcite, inulin, raffinose, glycerin, galactose and xylose, and tested in 48 to 72 hours with litmus.

For confirmation, the organisms were inoculated into Hess's semisolid medium containing Andrede as an indicator plus the following carbohydrates—dextrose, lactose, saccharose and mannite.

One per cent peptone lead acetate agar and three per cent peptone lead acetate agar were made according to directions given by Jordan.

Litmus milk, one per cent peptone gelatin, Dunham's peptone, nitrate broth were made according to directions in Standard Methods of Water Analysis.

Gram stains were made from cultures after 24 hours' growth upon an agar slant, using carbol gentian violet as the primary stain and counterstaining with an aqueous solution Bismarck brown.

The chromogenic power was determined by spreading a portion of a culture two weeks old upon white paper, as suggested by Winslow.

Blood agar plates were made by adding 3 cc. of whole defibrinated blood to 100 cc. of agar neutral to phenolphthalein. Sheep, rabbit and human blood were used. The sheep blood was all obtained from the same animal, three different rabbits were bled, and human blood was obtained from several individuals.

Milk plates were made by adding 10 cc. of milk to 100 cc. of agar. The agar was adjusted to + 2, + 1, 1, and — 1 to phenolphthalein.

The chlorimetric or indicator method was used in determining the hydrogen ion concentration. Buffers were made up according to Cole.[8] Methyl red, Phenol red and brom cresol purple were used as indicators as suggested by Clark and Lubs.[9]

The synthetic media used contained .5 per cent Bacto peptone (Digestive Ferments Company), .5 per cent dextrose and .5 per cent K_2HPO_4 titrated neutral to methyl orange. The media was sterilized at 10 pounds for 15 minutes, in order not to destroy the vitamines. After sterilization the hydrogen ion concentration of the broth was 7.3.

As previously mentioned, the first division of this work was a study of the fermentation reaction of all strains of staphylococci, especially with regard to dextrose, lactose, saccharose and mannite. As a matter of supplying additional information maltose, galactose, xylose, salacin are included in the report.

The summary of this data is included in table I.

Nomenclature was taken from Winslow's Systematic Relationship of *Coccaceæ*.

TABLE I.

CLASS 1—Organisms fermenting dextrose, lactose, saccharose and mannite.

No.	SOURCE.	Pigm.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Glyc.	Milk.*	Milk.**	Gel.	K_2PO_4 Broth.	Nitr.	Lead Acct.	Gram Stain.
5	Pus from ear	White	+	+	+	+	+	+	−	+	+	+	Pep	+	+	−	−	+ Albus.
30	Boil (Acalia)	O	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+ Aureus.
31	Boil (Johnson)	O	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+ Aureus.
38	Rabbit sore	White	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+ Albus.
48	Oyster	Clear	+	+	+	+	+	+	−	+	−	+	Pep	+	+	+	−	+ Albus.
17	H. Urine (Flu)	White	+	+	+	+	+	+	+	+	−	+	Pep	−	+	−	−	+ Albus.
49	Oyster	Clear	+	+	+	+	+	+	−	+	−	+	Pep	+	+	−	−	+ Albus.
16	L. Urine (Flu)	White	+	+	+	+	+	+	+	.....	−	+	Pep	+	−	−	−	+ Albus.
6	Milk	Y	+	+	+	+	+	+	−	−	−	−	+	+	+	+	−	+ Citreus.
12	Milk	White	+	+	+	+	+	−	+	−	−	+	Cog	+	−	+	+	+ Albus.
20	Rabbit Abscess	White	+	+	+	+	+	−	+	−	−	+	Cog	+	−	+	+	+ Albus.
27	Throat	White	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	+ Albus.
39	T. B. Infection	O	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+ Aureus.
40	Infected Tooth	White	+	+	+	+	+	+	−	−	+	+	+	−	−	−	−	+ Albus.
57	Sore Throat	O	+	+	+	+	+	+	−	−	−	+	+	+	.....	+	+	+ Aureus.
7	Milk	Y	+	+	+	+	+	+	+	−	−	+	Cog	+	−	+	+	+ Citreus.
3	Air	White	+	+	+	+	+	−	−	−	−	+	+	−	−	+	−	+ (?)
8	Milk	White	+	+	+	+	+	−	−	−	−	+	Pep	+	−	+	−	+ Albus.
9	Milk	O	+	+	+	+	+	−	−	−	−	−	Pep	+	+	+	+	+ Aureus.
55	Chronic Eye Infection	O	+	+	+	+	+	−	−	−	−	+	Pep	−	+	+	+	+ Aurantiacii.
58	J. P	W	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Variable Epidermidis.
59	Boil	O	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	Aureus.
61	—	O	+	+	+	+	+	+	+	−	−	+	.....	+	+	+	−	+ Aureus.
62	—	O	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+ Aureus.
63	—	O	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+ Aureus.
64	—	O	+	+	+	+	+	+	+	−	−	+	Pep	+	+	+	−	+ Aureus.
65	—	W	+	+	+	+	+	+	−	−	−	+	Cog. pep.	+	+	+	+	+ Albus.
66	—	W	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+ Albus.
67	—	W	+	+	+	+	+	−	+	−	−	+	+	+	+	+	+	+ Albus.
68	—	O	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+ Aureus.
72	Aureus	O	+	+	+	+	−	+	−	−	+	.....	Cog. pep.	+	+	+	−	+ Aureus.
76	Milk	O	+	+	+	+	+	+	−	−	−	+	Pep	+	+	+	+	+ Aureus.
78	Boil	O	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+ Aureus.
83	313	O	+	+	+	+	+	+	−	−	−	+	Cog	+	+	+	−	+ Aureus.
84	348	O	+	+	+	+	+	+	−	+	−	+	Pep	−	−	+	+	+ Aurantiacii.
85	457	O	+	+	+	+	+	−	+	−	−	+	−	−	+	+	+	+ Aurantiacii.
86	Boil	O	+	+	+	+	+	+	−	−	−	+	+	+	+	+	−	+ Aureus.
87	Pus	O	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+ Aureus.
88	Pus	O	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+ Aureus.
89	Pus	O	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+ Aureus.
90	Pus	O	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+ Aureus.
91	Pus	O	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+ Aureus.

O = Orange; W = White; Y = Yellow, C = Clear

TABLE I—CONTINUED.

CLASS 2—Organisms Fermenting Dextrose, Lactose, Saccharose but not Mannite.

No.	SOURCE.	Pigm.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Glyc.	Milk.	Milk.	Gel.	K_2PO_4 Broth.	Nitr.	Lead.	Milk plates, Gram stain.
29	Acne	White	+	+	+	—	+	+	+	—	.....	+	Cog	+	+	+	—	+ Epidemus.
34	Milk	White	+	+	+	—	+	+	+	—	.....	+	+	+	—	+	+	+ Epidemus.
37	Arm Infection	White	+	+	+	—	+	+	+	—	.....	—	—	+	+	+	—	+ Epidemus.
35	Milk	White	+	+	+	—	+	+	+	—	.....	+	+	+	+	—	+	+ Albus.
28	Acne	White	+	+	+	—	+	+	+	—	.....	+	+	+	+	+	—	+ Epidemus.
2	Skin of Nose	White	+	+	+	—	+	+	—	—	.....	+	Cog	+	+	+	—	+ Epidemus.
41	Butter	White	+	+	+	—	+	+	—	—	.....	+	+	—	—	+	—	+ Candidus.
43	Oleomargarine	Y	+	+	+	—	+	+	—	—	.....	+	+	—	—	+	—	— Luteus.
46	Butter	Y	+	+	+	—	+	+	—	—	.....	+	+	+	—	+	—	+ Citreus.
47	Infected Tooth	White	+	+	+	—	+	+	—	—	.....	+	Pep	+	—	+	—	+ Epidemus.
26	Throat	White	+	+	+	—	+	—	—	—	.....	+	+	—	—	—	+	+ Candidus.
52	Tonsil	O	+	+	+	—	+	—	—	—	.....	+	Pep	+	—	+	+	— Aureus.
33	Sneeze	White	+	+	+	—	+	—	—	—	.....	+	+	—	—	+	—	+ Candidus.
69	—	White	+	+	+	—	+	+	+	—	—	+	.....	+	—	—	+ +	+ Albus.
70	—	O	+	+	+	—	+	+	—	—	—	+	.....	+	—	—	+ —	+ Aureus.
77	Milk	Y	+	+	+	—	+	+	+	+	—	+	.....	+	—	+	+ +	+ Citreus.
80	Hamberger	Y	+	+	+	—	—	—	—	—	—	+	.....	+	—	—	— +	+ Flavius.
81	Nose	W	+	+	+	—	+	+	—	—	—	+	.....	+	+	+	— +	+ Epidermidis
82	Nose	W	+	+	+	—	+	+	—	—	—	+	.....	+	+	+	— +	+ Epidermidis

TABLE I—CONTINUED.

CLASS 3—Organisms Fermenting Dextrose and Saccharose but not Lactose or Mannite.

No.	SOURCE.	Pigm.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Milk.	Milk.	Gel.	Red.	Mit.	Lead.	Gram Stain.
4	Air	White	+	—	+	—	+	—	—	—	—	Pep	+	—	—	+	+ Albus.
53	Tonsil	O	+	+	+	—	+	—	—	—	—	—	.....	—	+	+	+
32	Sneeze	Y	+	—	+	—	+	—	—	—	—	—	—	—	—	—	— Luteus.
15	Scalp	Y	+	—	+	—	+	—	—	—	—	—	—	—	—	+	+ Luteus.

TABLE I—CONTINUED.

CLASS 4—Organisms not Fermenting Dextrose, Lactose, Saccharose or Mannite.

No.	SOURCE.	Pigm.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Milk.	Milk.	Gel.	Red.	Mit.	Lead.	Gram Stain.
21	Feces (Flu)	Light Yellow	—	—	—	—	—	—	—	—	—	—	+	—	+	—	+ Citreus.
25	Milk	Light Yellow	—	—	—	—	—	—	—	—	—	—	+	+	—	+	+ Flavus.
22	Feces (Flu)	Light Yellow	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+ Luteus.
54	Infected Tooth	White	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
50	Oyster	White	—	—	—	—	—	—	—	—	—	Pep	—	—	—	—	+
51	Sore Throat	Orange White	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+ Aurantiacus.

TABLE I—CONCLUDED.

CLASS 5—Irregular Organisms.

No.	SOURCE.	Pigm.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Milk.	Milk.	Gel.	Red.	Mit.	Lead.	Gram Stain.
11	Milk	Orange-White	+	—	+	+	+	—	—	—	—	Pep	+	+	+	+	+ Aureus.
23	Unknown	White	+	—	+	+	—	—	—	+	—	Pep	+	+	+	+	+ Epidermidis.
14	Pus from Rabbit	Light Yellow	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+ Luteus.
45	Air	Light Yellow	+	—	—	—	—	—	—	—	—	Pep	+	—	—	+	+ Flavus.

It will be observed that results in table I have divided all of the staphylococci into five classes. Class I, those staphylococci which ferment all four of the sugars, dextrose, lactose, saccharose and mannite; class II, those that ferment dextrose, lactose, saccharose, but are negative upon mannite; class III, those fermenting dextrose and saccharose but negative upon lactose and mannite; class IV, includes all staphylococci which failed to produce acid in any of the four sugars; and class V, includes four strains that are irregular.

It can readily be seen that there is no correlation between these classes in source, pathogenicity or pigmentation. For this reason, classifying staphylococci purely on fermentation reactions, disregarding pigment production and liquefaction of gelatin, does not seem to give a satisfactory classification.

The second phase was to assume pigmentation as a primary classification, using white, yellow and orange, and subdividing each of these, making use of the fermentation reaction of the sugars. In doing this, I have assumed that dextrose, lactose and mannite are of importance in the order named and have developed the classifications which are shown in table II.

Again it can be seen that there is no apparent correlation between these fermentation reactions and pigmentation or source or pathogenicity.

Subdivision 3 of this problem comprises an application of the phenomena of hemolysis to subdivision of various pigmented types of staphylococci. There are various and conflicting statements in literature as to most suitable kind of blood for determining hemolysis by staphylococci. It is quite generally recommended that a washed suspension of red blood cells be used, but for routine laboratory work this process is not ordinarily followed, largely because of the lack of facilities and the desire for speed. In order to duplicate ordinary laboratory methods, I have made use of blood agar prepared by adding defibrinated blood to melted agar cooled to 45° C.

Before attempting this work I tried the hemolytic properties of these organisms for rabbit, sheep and human bloods to determine which gave the most positive and fairly consistent results. These are embodied in table III.

TABLE II.

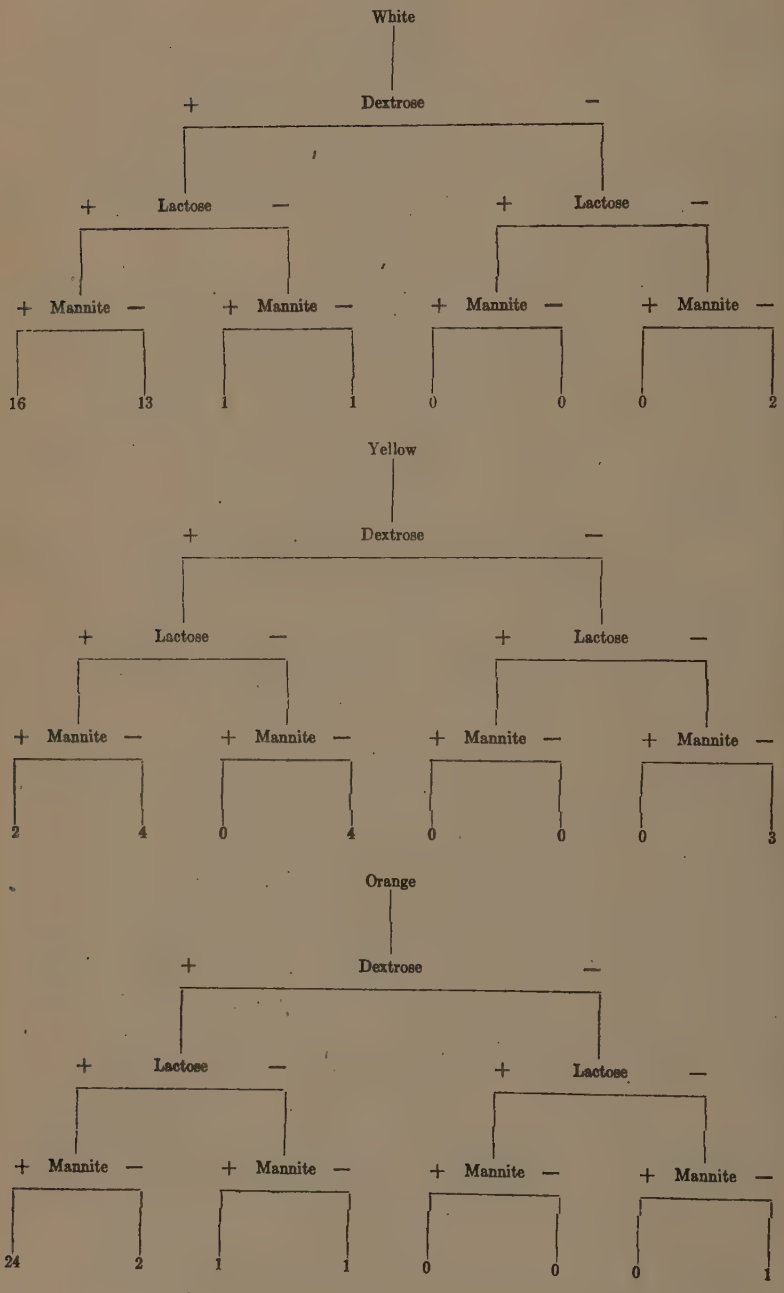

White
+ Dextrose −
+ Lactose − + Lactose −
+ Mannite − + Mannite − + Mannite − + Mannite −
16 13 1 1 0 0 0 2
Yellow
+ Dextrose −
+ Lactose − + Lactose −
+ Mannite − + Mannite − + Mannite − + Mannite −
2 4 0 4 0 0 0 3
Orange
+ Dextrose −
+ Lactose − + Lactose −
+ Mannite − + Mannite − + Mannite − + Mannite −
24 2 1 1 0 0 0 1

TABLE III.

Source.	Strain and Group No.	Pigm.	Rabbit Blood + Hemolysis —		Sheep Blood + Hemolysis —		Human Blood + Hemolysis —		Milk Plates + Proteolysis —	
			+	—	+	—	+	—	+	—
Air	3[1]	White		—		—		—		—
Milk	8[1]	White		—	+		+		+	
Milk	12[1]	White		—		—		—		—
Urine F	17[1]	White		—		—		—		—
Rab. Absc.	20[1]	White		—	+		+			—
Throat	27[1]	White	+		+		+		+	
Rab. Sore	38[1]	White	+			—	+			—
Urine F	16[1]	White		—		—	+		+	
Inf. Tooth	40[1]	White	+		+		+			—
Pus—ear	5[1]	White		—		—		—	+	
Oyster	48[1]	Clear		—		—		—	+	
Oyster	49[1]	Clear		—	+		+		+	
—	65[1]	White		—	+		+		+	
G. P. Autopsy	58[1]	White	+		+		+			—
—	66[1]	White	+			—	+			—
—	67[1]	White		—		—	+		+	
Acne	29[2]	White		—		—		—	+	
Milk	34[2]	White	+		+		+		+	
Arm. Inf.	37[2]	White		—		—		—	+	
Milk	35[2]	White	+		+		+			—
Acne	28[2]	White		—		—		—	+	
Skin—nose	2[2]	White		—		—		—		—
Butter	41[2]	White		—		—		—	+	
Inf. Tooth	47[2]	White		—	+		+		+	
Throat	26[2]	White	+			—		—		—
Sneeze	33[2]	White		—		—		—		—
—	69[2]	White	+		+		+		+	
None	81[2]	White		—		—		—	+	
None	82[2]	White	+			—	+		+	
—	54[3]	Lost								
Oyster	50[3]	Lost		—		—		—		—
	4[4]	Lost								
	23 ir	Lost								
Milk	6[1]	Y. White	+			—	+			—
Milk	7[1]	Y. White		—		—		—		—
Oleomargarine	43[2]	Y. White		—	+		+		+	
Butter	46[2]	Y. White		—		—		—		—
Milk	77[2]	Yellow		—	+		+		+	
Hamburger	80[2]	Yellow		—		—	+		+	
Feces	21[3]	Yellow	+		+		+		+	
Milk	25[3]	Yellow	+		+		+		+	
Feces	22[3]	Yellow	+		+		+		+	
Rab. Pus	14[3]	Yellow		—		—		—		—
Air	45[3]	Yellow	+		+		+		+	
Scalp	15[4]	Yellow		—		—	+		+	
Milk	9[1]	Orange		—		—		—	+	
Boil	30[1]	Orange		—	+		+			—
Boil	31[1]	Orange		—		—		—	+	
T. B. Inf.	39[1]	Orange		—		—	+			—
Sore Throat	57[1]	Orange		—	+		+		+	
Eye	55[1]	Orange	+		+		+		+	
Boil	59[1]	Orange		—	+		+		+	
—	61[1]	Orange	+		+		+		+	
—	62[1]	Orange		—	+		+		+	
—	63[1]	Orange	+		+		+		+	
—	64[1]	Orange	+		+		+			—
—	68[1]	Orange		—		—	+		+	
Lab. St.	72[1]	Orange	+		+		+			—
Boil	78[1]	Orange		—	+		+		+	
Aureus	83[1]	Orange		—		—	+			—
Aurientiacus,	84[1]	Orange	+		+		+		+	
Aurientiacus,	85[1]	Orange		—	+		+		+	
Boil	86[1]	Orange	+		+		+			—
Boil	87[1]	Orange	+		+		+		+	
Boil	88[1]	Orange	+		+		+		+	
Boil	89[1]	Orange	+		+		+		+	
Boil	90[1]	Orange	+		+		+		+	
Boil	91[1]	Orange	+		+		+		+	
Milk	76[1]	Orange		—		—	+			—
Tonsil	52[2]		+		+		+		+	
—	70[2]	Orange		—		—		—		—
Sore Throat	51[3]	Orange		—	+		+			—
Milk	11 ir	Orange	+		+		+		+	
Tonsil	53[4]	Orange	+		+		+		+	

It is quite evident that human blood gave the most positive results.

I decided, as mentioned above, to use pigmentation as the primary method of division and blood agar plates secondarily, subdividing each of these into hemolytic and nonhemolytic staphylococci, and the fermentation reactions as described in table II were made use of for further subdivision. The results of this are summarized in table IV.

It will be observed that the white staphylococci were evenly divided between hemolytic and nonhemolytic strains, 16 strains were hemolytic and 14 strains were nonhemolytic. This condition shows a gradual change as you go through the yellow and orange staphylococci. For example, out of 13 yellow staphylococci, one was lost before hemolytic properties were determined and of the remaining 12, 9 were hemolytic and 3 were nonhemolytic. Among the orange staphylococci, 26 strains were hemolytic and 3 nonhemolytic. Of these 26 hemolytic orange staphylococci, 19 were from the animal body as compared with one among the three of the nonhemolyzers. Of the 19 from the animal body, 16 were positive in all sugars. Among the yellow, only one was from the animal body and that one was nonhemolytic and fermented dextrose but not lactose or mannite. Among the white hemolytic staphylococci, 7 were from the animal body and of these 7, 6 fermented all sugars. Among the 14 nonhemolyzers, 2 were from the animal body. This suggests that in general staphylococci associated with the animal body seem to be hemolyzers. The history of organisms obtained from the air and various foods is not known further than the source mentioned.

As the fourth phase of this problem, we have attempted to study a possible classification of staphylococci, making use of pigment as a primary division and next the ability of the staphylococci to produce proteolysis or conversely failure to produce proteolysis. This is followed by making use of carbohydrates as in previous tables. It will be observed that the only difference between this and the third phase is that proteolysis is substituted for hemolysis.

Very little work has been published showing the use of milk agar plates in the attempt to classify any kinds of bacteria at all. As a preliminary it was found necessary to determine the optimum reaction of media for proteolysis. Accordingly, studies were made on milk plates + 2, + 1, 1, and — 1 to phenolphthalein. The results are summarized in table V.

TABLE IV.

White
Hemolysis
+ —
Dextrose
Lactose
Mannite
11 5 0 0 0 0 0 0 5 8 0 0 0 0 0 1
Yellow
Hemolysis
1 3 0 1 0 0 0 4 1 1 0 1 0 0 0 0
Orange
Hemolysis
22 1 1 1 0 0 0 1 2 1 0 0 0 0 0 0

TABLE V.—Effect of Reaction of Media on Proteolysis on Milk Agar Plates.

Pigment.	Total number of strains.	Reaction of media and number of strains showing proteolysis.															
		+ 2 to phenolphthalein.				+ 1 to phenolphthalein.				1 to phenolphthalein.				1 to phenolphthalein.			
		None.	Trace.	Fair.	Good.	None.	Trace.	Fair.	Good.	None.	Trace.	Fair.	Good.	None.	Trace.	Fair.	Good.
White.....	30	18	4	0	8	16	6	.....	8	14	4	2	10	15	5	1	9
Yellow.....	12	7	2	0	3	7	2	.....	3	6	0	0	6	6	4	0	2
Orange.....	29	15	6	3	5	14	4	6	5	12	3	9	5	10	11	5	3

It is quite evident that the best reaction was neutral to phenolphthalein, the end point was a pronounced end point and corresponded to a P_h of about 8.8.

Applying this in the same manner as blood agar plates in table IV, I have summarized the data in table VI.

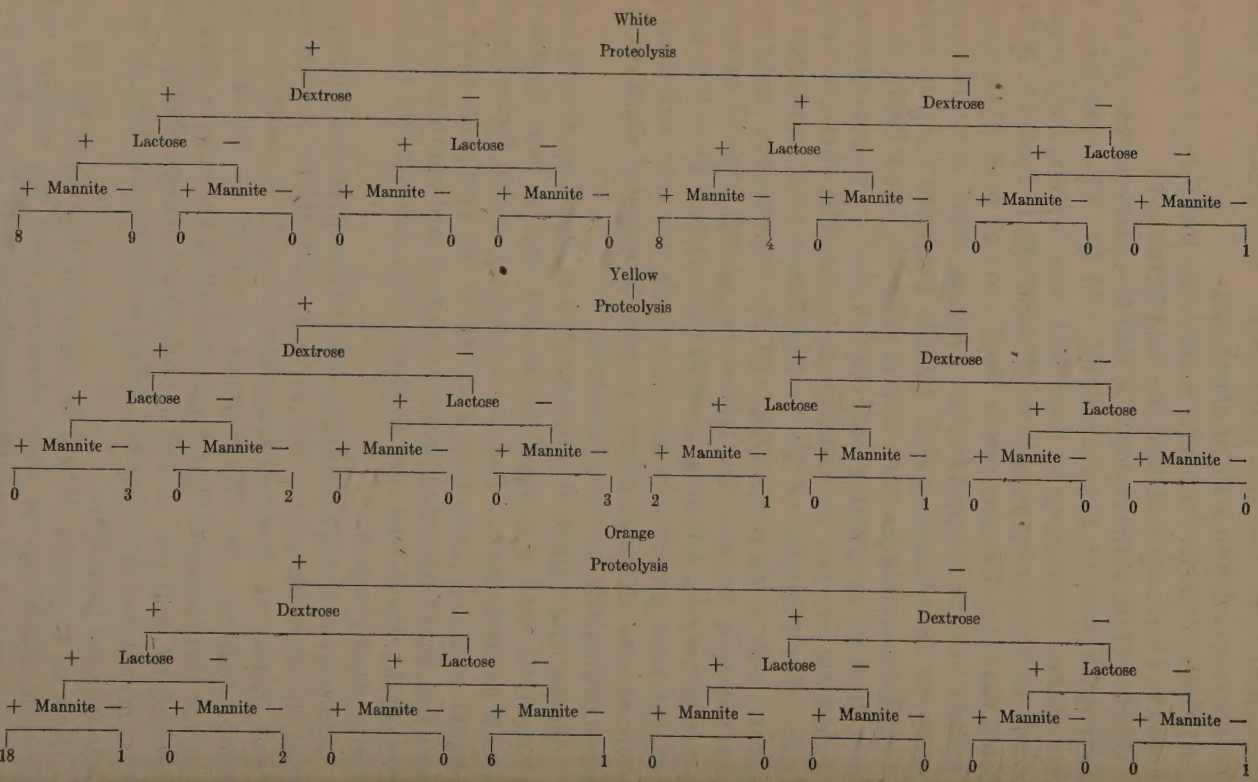
Of the white staphylococci, it will be observed that 17 were proteolytic and 12 nonproteolytic. Of the 17 proteolytic, it was rather interesting to note that only 3 were body organisms. In comparing results with hemolysis in table IV, it was noted that on milk agar plates there were 17 proteolytic staphylococci and 12 nonproteolytic, whereas there were 16 hemolyzers to 13 nonhemolyzers. While the total number found proteolytic compares very closely with the total found hemolytic, it is an interesting observation that organisms that are proteolytic are not necessarily the same ones that are hemolytic. For example, of the 11 hemolyzers that fermented all sugars, only 8 are proteolytic. Of the 9 proteolytic organisms that ferment dextrose and lactose but do not ferment mannite, 5 are hemolytic, 4 failing to show hemolysis. Thus it is quite evident that proteolysis and hemolysis are not consistent in their actions although about the same number of staphylococci were proteolytic as were hemolytic.

Among the yellow staphylococci it is observed that 8 were proteolytic and 4 nonproteolytic and that 9 were hemolytic and 3 nonhemolytic. The one hemolyzer which fermented all sugars was not proteolytic and one of the two proteolytic organisms that fermented dextrose, lactose and mannite was not hemolytic, which was very similar to the observations made on white staphylococci.

Among the orange staphylococci, it was previously observed that 26 were hemolytic and three nonhemolytic. Using milk agar plates, we observed that there were 21 proteolytic and 8 nonproteolytic. In other words, 4 of 22 of the hemolytic orange staphylococci that fermented all of the sugars were not proteolytic and one of the 2 proteolytic orange staphylococci that fermented dextrose but failed to ferment lactose or mannite was not hemolytic.

Now as to source, it will be observed that 14 of the 21 proteolytic staphylococci were obtained from the animal body. The percentage of organisms associated with the animal body was greater with the nonproteolytic than with the nonhemolytic orange staphylococci.

TABLE VI.


White
Proteolysis + −
Dextrose + −
Dextrose + −
Lactose + −
Lactose + −
Lactose + −
Lactose + −
Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + −
8 9 0 0 0 0 0 0 8 4 0 0 0 0 0 1
Yellow
Proteolysis + −
Dextrose + −
Dextrose + −
Lactose + −
Lactose + −
Lactose + −
Lactose + −
Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + −
0 3 0 2 0 0 0 3 2 1 0 1 0 0 0 0
Orange
Proteolysis + −
Dextrose + −
Dextrose + −
Lactose + −
Lactose + −
Lactose + −
Lactose + −
Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + − Mannite + −
18 1 0 2 0 0 6 1 0 0 0 0 0 0 0 1

The fifth subdivision of this paper has to do with the action of all staphylococci upon lead acetate agar. A summary of this data is embodied in table I. It will be observed that, with two exceptions, all staphylococci isolated from pus or boils blackened lead acetate agar. I doubt, however, that this could be depended upon to denote pathogenicity.

In regard to the sixth subdivision of the paper applying to the various hydrogen ion concentrations, I hope to do more extensive work in the future. I selected 6 from class 1, table I, 6 from class 2, 2 from class 3, and 4 from class 4, and grew them in dextrose dipotassium phosphate broth, as described in the paragraph on technique, and determined the hydrogen ion from day to day for a period of five days.

These results suggest the possibility of dividing staphylococci into subdivisions depending upon the limiting P_h. This is analogous to the attempt to subdivide the coliærogenes group. It might be of some value if used with pigment production as a basis of classification and the high ratio determined for white, yellow and orange separately.

I have also considered the value of the group number system as suggested in the descriptive chart of the American Association of Bacteriology, but have decided not to include the various group numbers of the various staphylococci in question.

SUMMARY AND CONCLUSIONS.

That disregarding pigmentation and liquefaction of gelatin staphylococci may be arranged into five types according to their ability to ferment dextrose, lactose, saccharose and mannite. These types do not correlate with any other observed characteristics such as source, pathogenicity, pigmentation or liquefaction of gelatin. It would seem that this method of classifying staphylococci would only lead to confusion and offers nothing of basic value.

That while routine laboratory work might warrant only the data on morphology, gram stain, type of growth and pigment production on plain agar slants, yet it would seem advisable, at least from the standpoint of comparison when reporting upon staphylococci in the literature, to follow some such plan as follows: Gram stain, pigment production, liquefaction of gelatin, action on blood agar plates where kind of blood, amount and P_h of medium are given, and the fermentation reaction in dextrose, lactose and mannite. Instead of blood agar plates it

would seem that for comparison milk agar plates might equally well be substituted and perhaps prove equally reliable. In either proteolysis or milk agar plates or hemolysis, it is apparently important to have an optimum and known hydrogen-ion concentration in the medium. This is very easily a source of discrepancies. The blackening of lead acetate agar might also be worth including.

There does not seem to be any uniform correlation between the property of proteolysis of milk agar plates and hemolysis on blood agar plates.

Apparently most staphylococci from the animal body are hemolytic.

Contrary to frequent statements in the literature, human blood seemed to be superior to either rabbit or sheep blood.

As might well be expected, hydrogen-ion determinations show that staphylococci can rightly be grouped into at least two groups with respect to some one indicator such as methyl red, and into more groups if desired. I do not know that this is consistent or will prove of value.

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